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Review Article

Molecular Detection of INH Susceptibility is also Required for Improved Performance in Pulmonary Tuberculosis

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Abstract

Diagnosis is the weakest link in our efforts to eliminate Tuberculosis. If detected early, screened for drug resistance and fully treated with appropriate multidrug regimen, TB can be cured. The diagnostic methods since 1882 had been smear microscopy. Subsequently culture was added to the diagnostic modalities. While smear was insensitive needing atleast 10,000 bacteria for reliable detection, culture was slow, taking weeks. The assessment and recommendation of Gene Xpert, a cartridge based molecular method which not only detected MTBC but also reported on Rifampicin susceptibility was a major improvement. Initially Rif resistant was thought to be a good surrogate marker for MDR TB. Recent evidence indicate that was not correct and may have been counter productive. If the isolate was INH susceptible but Rif mono resistance and as per recommendation treated as MDR TB, then INH, an excellent bactericidal drug would have been with-held. On the other hand if INH monoresistant (IMR) was present and patient was treated with standard regimen, then for four months in the continuation phase, patient would receive monotherapy which would encourage emergence of MDR TB, failure of treatment, relapse or death.

Keywords: Diagnosis; WHO; MTBC

WHO has recently evaluated and approved four new molecular methods which not only detect MTBC but also simultaneously report on both INH and Rif susceptibility. India TB 2023 report indicates that in a large majority of patients (78.8%) the isolates were susceptible to both INH and Rif, therefore one of these should be used for screening as well simultaneous molecular DST, so that patients were rapidly placed on the appropriate treatment leading to not only their excellent response to treatment but also decrease in spread of infection in the society.

Tuberculosis has been the leading cause of death globally. If detected early, screened for drug resistance and fully treated with appropriate multidrug regimen, TB can be cured. But diagnosis is the weakest link in the TB continuum of care. The current diagnostic methods, such as sputum smear microscopy, have limitations in terms of sensitivity and the ability to detect drug resistance. This leads to a significant diagnostic gap, where many cases of TB go undiagnosed and untreated. Improving the diagnostic process is crucial in order to effectively treat and eliminate tuberculosis [1].

The main challenges in diagnosing tuberculosis (TB) include: 1. Limited access to diagnostic tools: Many low- and middle-income countries (LMICs) lack access to reliable and accurate diagnostic tools for TB. This hinders early detection and timely treatment. 2. Low sensitivity of smear microscopy: Smear microscopy, which has been the frontline diagnostic test for TB, has limitations in terms of sensitivity, especially in cases of HIV co-infection, children, and extrapulmonary TB. It also cannot detect drug resistance. 3. Delayed diagnosis: Due to the limitations of existing diagnostic methods, there are often delays in diagnosing TB. This can lead to further transmission of the disease and poorer health outcomes for patients. 4. Lack of drug resistance testing: Drug-resistant TB is a growing concern, but access to drug resistance testing is limited in many settings. This results in undiagnosed cases of drug-resistant TB and inadequate treatment. 5. Complex quality assurance systems: Microscopy, the traditional diagnostic method, requires complex quality assurance systems to maintain performance. This adds to the challenges of implementing and sustaining accurate TB diagnosis. 6. Limited population coverage: The current diagnostic methods may not reach all individuals who need testing, particularly those in remote or marginalized communities. This leads to underdiagnosis and underreporting of TB cases. 7. Fragmented healthcare systems: In some settings, healthcare systems are fragmented, making it difficult to ensure coordinated and comprehensive TB diagnosis and care [1].

The COVID-19 pandemic has had a significant impact on the diagnosis of tuberculosis (TB). Before the pandemic, there was already a diagnostic gap in TB, with many cases going undiagnosed. However, the pandemic further exacerbated this gap. TB services

were severely affected, many personnel and infrastructure used for TB diagnostics was diverted towards SARS Co 2 diagnosis, leading to a decrease in the number of TB cases diagnosed and notified to national TB programs [1].

India TB report 2023 indicate that in 2022 the National TB elimination program (NTEP) put 22,48,649 (95.3%) on treatment and obtained 85% success rate. 4.2% dies, 2.6% were lost to follow up and there were 1.9% treatment failure. Bacteriologically confirmation was obtained in 12,32,149 (51%) of pulmonary tuberculosis cases. Valid rapid DST for Rif Resistance was performed in 9,38, 217 (76%) cases and resistance was detected in 63,801 patients. Using LPA 1, INH monoresistance was detected in 15,953 cases. Of the 2,88,549 (94.4%) of samples which were positive for TB by molecular methods, 2,40,906 (78.8%) were susceptible classified as DS -TB and 20,125(7%) as MDR-TB. INH Monoresistance (IMR) was detected in 20,463 (7.1%) while rifampicin monoresistance was detected in 7055 (2.4%). Using LPA 2, fluoroquinolone resistance was detected in 29.8% samples tested only 1.6% were resistant to second line injectables. Of the 1,39,14,910 presumptive TB patients, TB diagnosis was offered through 23,038 Microscopy centre and 6,31,683 (4.5%) were diagnosed as TB (smear microscopy is relatively insensitive as it requires presence of 10,000 bacteria in the sample before reaching detectable level and hence WHO in 2020 reiterated its previous guidelines as to use molecular methods instead of smear for case finding). Gene Xpert (CBNAAT) was used on 23,65,000 (22%) samples and TrueNAT on 34,83,000 samples and 5,25,088 cases were detected by Gene Xpert and 5,29,196 (15%) by TrueNat. Rifampicin resistance was detected in 42,026 (8%) by Gene Xpert and 21,659(4%) by TrueNat, no explanation has been offered between these two results. First line LPA was conducted on 3,09,719 positive samples (out of 10,54,284 eligible samples, all positive samples as per NEPT algorithm) and TB was confirmed in 2,88,549 (94.4%) and 2,40,906 (78.8%) were found to be susceptible to both INH and Rif., 20,125 (7%) were MDR TB, 20,463 (7.1%) were IMR and 7055 (2.4%) were Rif monoresistant. On testing MDR TB strains with second line LPA, fluoroquinolone resistance was detected in 29.8% while resistance to seconline injectable aminoglycosides had only 1.6% resistance (WHO no longer recommends use of SLI for treatment) [2].

Rifampicin has been considered a reliable surrogate marker for multidrug-resistant tuberculosis (MDR-TB). Rifampicin is one of the most effective drugs used to treat tuberculosis, and resistance to rifampicin is strongly associated with resistance to other key drugs used in MDR-TB treatment. Therefore, testing for rifampicin resistance is an important indicator of MDR-TB [3].

INH, first synthesized in 1912 in Prague, is an effective first-line drug for the treatment of active TB disease. A prodrug, INH is activated by the catalase-peroxidase KatG of *Mycobacterium tuberculosis* (M. tb). Following this, it binds InhA, an enoyl-acyl carrier protein reductase and so blocks fatty (mycolic) acid synthesis, a key component of the bacterial cell wall. In rapidly dividing bacteria INH is bactericidal, in slower dividing bacteria bacteriostatic. The drug is thought to provide a high initial kill at the start of active TB treatment, after which RMP largely takes over in terms of bactericidal activity and RMP and pyrazinamide (PZA) act as sterilizing drugs. From its earliest use as monotherapy for TB disease in the 1950s, rapid and frequent development of resistance to INH was reported. Such observations regarding INH and other drugs emphasized the need for combination regimens. INH, streptomycin (STM) and p-aminosalicylic acid (PAS) thus became the standard regimen for many years before the development of the current short course of two months of INH, RIF, PZA and ethambutol (EMB), followed by four months of INH and RIF [4].

For decades, no drug-susceptibility testing (DST) for any drug was done unless patients failed first-line therapy or had risk factors for drug-resistant TB (DR-TB). Simply put, we chose to ignore the problem. When the TB world woke up to the need for universal DST and included it as a key goal in the End TB Strategy released in 2015, the focus became rapid testing for rifampicin resistance (RR) as a means of achieving universal DST. Novel technologies such as Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) were rolled out in 2010, but the technology did not include INH-resistance testing [3].

WHO recommended that Xpert MTB/RIF should be used rather than conventional microscopy, culture and DST as the initial diagnostic test in adults suspected of having MDR-TB or HIV-associated TB. Initial data had shown that Xpert MTB/RIF detected some rifampicin-resistant strains that were identified as susceptible by phenotypic DST. Sequencing these discordant results were resolved in favour of Xpert MTB/RIF, and patients missed by phenotypic DST had poor treatment outcomes on first-line treatment [5,6].

Using Xpert MTB/RIF to diagnose pulmonary TB and rifampicin resistance in adults a total of 27 unique studies involving 9558 participants were included in the systematic review. Two of the 27 studies were multicentre international studies (one with five distinct study centres and the other with six). Two of the 27 studies evaluated Xpert MTB/RIF in primary care clinics where the results were used to begin treatment on the same day. Sixteen studies (59%) were performed in low-income or middle-income countries [5].

When used to detect rifampicin resistance, Xpert MTB/RIF achieved a pooled sensitivity of 95% (95% CrI, 90-97%), (17 studies, 555/2624 total specimens) and a pooled specificity of 98% (95% CrI, 97-99%), (24 studies, 2414 specimens) [5].

All TB cases diagnosed by Xpert MTB/RIF and found to be rifampicin-resistant were recommended to be entered in the register at the Basic TB Management Unit and in laboratory registers

as rifampicin resistant TB (denoted as RR-TB) and also noted as Xpert MTB/RIF-positive with rifampicin resistance. If isoniazid resistance was detected by conventional or molecular techniques, the case was to be registered as MDR-TB [5].

The question whether there was need to detect Isoniazid Resistance in addition to Rifampicin Resistance in Diagnostic Tests for Tuberculosis was addressed by a computer model [6]. Compared to the TB test alone and assuming treatment of all diagnosed MDR cases, the TB+RIF test reduced the prevalence of MDR-TB among all TB cases from 5.5% to 3.8% (30.6% reduction, 95% uncertainty range, UR: 17-54%). Despite using liberal assumptions about the impact of INH-monoresistance on treatment outcomes and MDR-TB acquisition, expansion from TB+RIF to TB+RIF/INH lowered this prevalence only from 3.8% to 3.6% further (4% reduction, 95% UR: 3-7%) and INH-mono resistant TB (IMR TB) from 15.8% to 15.1% (4% reduction, 95% UR). It was concluded that the impact of detecting isoniazid resistance in addition to rifampicin resistance on MDR-TB rates was uncertain and might have minimal impact on transmission of TB, MDR-TB, and isoniazid-monoresistant TB [7].

That IMR was prevalent was known for many decades. In the Eastern European region, the estimated percentage of incident tuberculosis (TB) cases with isoniazid resistance was 44.9%. In all other regions combined, the estimated percentage of incident TB cases with isoniazid resistance was 13.9%. The levels of isoniazid resistance among new TB cases were 33.5% globally, and among retreatment cases, it was 61.4%. However, it is important to note that these trends were not consistent across all settings, and data availability for monitoring isoniazid resistance was limited in many countries. But there were reports that IMR did not adversely affect response to treatment.

Modern experience with the treatment of INH monoresistance (IMR) was initially described in a 1986 summary report of British Medical Research Council TB treatment trials performed in Africa, Hong Kong, and Singapore. In this report, 72/72 (100%) IMR patients achieved treatment success (notably, all were treatment-naive) when treated for at least 6 months with 4 or more antimycobacterial drugs. A more contemporary retrospective study from San Francisco essentially confirmed these results, showing rates of treatment failure with IMR that were not different than DS-TB when treated with at least 6 months of four-drug therapy. However, other studies, primarily retrospective, from high-burden, lower-resource settings have shown increased risk for treatment failure with IMR treatment compared with DS-TB. A limitation of these retrospective studies was the incomplete description of treatment regimen and confounding by indication (i.e., drug-susceptibility testing performed preferentially in patients failing treatment). The implication of this research was that the phenotype of IMR may be highly clinically significant and more appropriately conceptualized as a precursor to MDR-TB, and as such, required intensified diagnostic and therapeutic approaches [8-10].

Paradoxically, the broad global rollout of the real-time polymerase chain reaction platform, Gene Xpert MTB/RIF, may have led to further selection for isoniazid-resistant MTB isolates and emergent MDR-TB, as GeneXpert MTB RIF diagnosed rifampin but not isoniazid resistance. Routine testing of MTB isolates for all relevant drugs should not be regarded as costly extras but, rather, as critical steps for overall drug-resistant TB control [11]. Indian algorithm in 2017 recommended that all isolates should be subjected to LPA 1 for determination of INH susceptibility and Rif resistant isolates to LPA 2 for FQ susceptibility (injectables are no longer recommended for treatment). But unfortunately there is both a time delay of weeks if the test is done and in many cases not performed [12].

That the strategy of using RR as a surrogate marker for MDR-TB may be suboptimal was reported by Bisimiwa., *et al*. [13] from South Africa. They reported that while Xpert had a sensitivity of 100.0% (95% CI, 92.1-100.0) for detecting RIF resistance but a positive-predictive value of only 61.6% (95% CI, 49.5-72.8) for MDR-TB. They cautioned that that relying on RIF resistance in isolation, without ascertainment of INH resistance, can lead to suboptimal treatment of INH- or RIF-monoresistant TB.

Peru (15,16,17)

The 2019 WHO guideline recommends that patients with INH-resistant and RIF susceptible TB be treated with a 6-month regimen composed of RIF, ethambutol (EMB), pyrazinamide (PZA), and levofloxacin. Patients with INH-monoresistant TB who are treated with a 6-month first-line TB regimen (2-month INH-RIF EMB-PZA/4-month INH-RIF) have higher risks of treatment failure, relapse, and acquiring additional resistance than those with drug-susceptible TB. Conversely, patients with confirmed low-level or no INH resistance (RIF-monoresistant TB) would benefit from the inclusion of INH in their treatment regimens [18].

Gegio., *et al*. (2017) [19] identified 19 cohort studies and 33 trials with 3744 patients with isoniazid-resistant tuberculosis and 19 012 patients with drug-sensitive disease. The pooled rates of failure or relapse, or both, and acquired drug resistance with all drug regimens were 15% (95% CI 12-18) and 3·6% (2-5), respectively, in patients with isoniazid-resistant tuberculosis and 4% (3-5) and 0·6% (0·3-0·9) in those with drug-sensitive tuberculosis. Of patients with initial isoniazid-resistant tuberculosis with acquired drug resistance, 96% (93-99) had acquired multidrug-resistant disease. Treatment of isoniazid-resistant tuberculosis with the WHO standard regimen for new patients resulted in treatment failure, relapse, and acquired multidrug resistance in 11% (6-17), 10% (5-15) and 8% (3-13), respectively; treatment with the standard WHO regimen for previously treated patients resulted in treatment failure in 6% (2-10), relapse in 5% (2-8), and acquisition of multidrug resistance in 3% (0-6). For patients with drug-sensitive disease treated with the standard retreatment regimen the rates were 1% (0-2), 5% (4-7), and 0·3% (0-0·6).

The global prevalence of rifampicin resistance is well documented, occurring in 3.4% (95% CI 2.5%-4.4%) of new TB patients and 18% (95% CI 7.6%-31%) of previously treated TB patients in 2018, whereas the prevalence of isoniazid resistance at global and regional levels is less understood. In 2018, the World Health Organization (WHO) recommended a modified 6-month treatment regimen for people with isoniazid-resistant, rifampicin-susceptible TB (IMR-TB), which included rifampicin, pyrazinamide, ethambutol, and levofloxacin [19].

Aggregated data reported to WHO from 156 countries or territories in 2002-2018 to estimate the prevalence of IMR-TB among new and previously treated TB patients. The global prevalence of isoniazid resistance among new tuberculosis (TB) patients was 7.4%, and among previously treated TB patients, it was 11.4%. There was wide variability in the prevalence of isoniazid resistance and multidrug-resistant TB (MDR-TB) between countries within the same region, indicating the influence of setting-specific factors on the emergence and spread of resistance. The prevalence of isoniazid resistance without rifampicin resistance (IMR-TB) was higher than the global estimate of rifampicin resistance, suggesting that screening for potential IMR-TB may be important in addition to rifampicin-resistant TB. The quality of phenotypic and genotypic testing for rifampicin and isoniazid may vary between countries, leading to misclassification of cases and potentially biasing estimates of resistance prevalence. It became obvious that many patients with IMR-TB would be missed by current diagnostic algorithms driven by rifampicin testing, highlighting the need for new rapid molecular technologies to ensure access to appropriate treatment and care [19].

Salaam-Dreyer., *et al*. [20] reported that RR TB was not the same as MDR TB. In South Africa RR mono resistance but INH susceptible accounted for 38% of isolates examined by Next Generation Sequencing (NGS). Significant difference in distribution of rpoB mutations conferring resistance to the isolate was found between RR isolates and MDR TB isolates. Mutations associated with high level Rif resistance were found in MDR TB (811/889) vs 162/130 among RR mono resistance isolates. rpoB L430P mutation, conferring low level RR was identified in 32/230 (13.9%) isolates vs 1.1% in MDR TB isolates. Amongst these 10 isolates 7 were phenotypically susceptible using a critical conc of 0.5 ug/ml. These data suggested that evolution of RR isolates were different from MDR isolates, with HIV coinfection play a role in RR isolates, which were susceptible to all other ATT drugs.

Mchakie., *et al*. [21] reported Resistance to either rifampicin or isoniazid sub-optimally predicts MDR-TB. Despite having high sensitivity and specificity, the positive predictive value of rifampicin was only 62.1% and for isoniazid was 78.3%, suggesting that if either was tested in isolation both could result in false positives MDR-TB cases, resulting into patients being unnecessarily subjected to the more toxic and expensive second-line anti-TB drugs, which were less effective compared to first-line anti-TB drugs.

It has now become clear that simultaneous detection of isoniazid (INH) and rifampicin (RIF) susceptibility in tuberculosis (TB) diagnosis would have a significant impact on the diagnosis and treatment of the disease. The key benefits being: 1. Early detection of drug resistance: Simultaneous detection of INH and RIF susceptibility would allow for the early identification of drug-resistant TB cases. This was crucial because drug-resistant TB requires different treatment regimens compared to drug-susceptible TB. Identifying drug resistance early would help in initiating appropriate treatment promptly. 2. Personalized treatment: Knowing the drug susceptibility profile of a TB patient enables healthcare providers to tailor the treatment regimen accordingly. With simultaneous detection of INH and RIF susceptibility, healthcare providers could choose the most effective combination of drugs to treat the specific drug-resistant strain, improving treatment outcomes. 3. Preventing the spread of drug-resistant strains: Identifying drug-resistant TB cases early not only benefits the individual patient but also helps prevent the spread of drug-resistant strains in the community. By promptly initiating appropriate treatment, the transmission of drug-resistant TB can be reduced, contributing to TB control efforts. 4. Avoiding ineffective treatments: Without simultaneous detection of INH and RIF susceptibility, there was a risk of prescribing ineffective treatments to patients with drug-resistant TB. This can lead to treatment failure, prolonged illness, and increased transmission of drug-resistant strains.

The available methods for detecting isoniazid (INH) susceptibility include: 1. Phenotypic Drug Susceptibility Testing (DST): This method involves culturing the *Mycobacterium tuberculosis* (MTB) bacteria and exposing them to different concentrations of isoniazid to determine their susceptibility. 2. Molecular Assays: Several molecular assays have been developed to detect specific genetic mutations associated with isoniazid resistance. These assays target specific genes, such as katG and inhA, which are known to be associated with INH resistance.3. Sequencing: DNA sequencing techniques can be used to identify specific mutations in the genes associated with isoniazid resistance. This method provides detailed information about the genetic changes in the MTB bacteria. 4. Line Probe Assays (LPAs): LPAs are molecular assays that use DNA probes to detect specific genetic mutations associated with drug resistance. These assays can simultaneously detect multiple resistance mutations, including those related to isoniazid resistance.

Molecular tests for tuberculosis (TB) have the capability to detect gene mutations associated with drug resistance. These tests can identify specific mutations in mycobacterial genes that are associated with resistance to anti-TB drugs. This information allows clinicians to tailor effective TB treatment by selecting appropriate drugs based on the patient's drug resistance profile. The World Health Organization (WHO) has recommended the use of molecular nucleic acid amplification tests (NAATs) for TB detection, as they can accurately detect TB and also identify drug resistance mutations. These molecular tests play a crucial role in improving

the quality of care for TB patients by providing rapid and accurate results, including drug susceptibility testing [22].

When selecting molecular rapid diagnostic tests for tuberculosis, there are several key factors to consider: 1. National policies and goals: Consider the alignment of the diagnostic tests with national policies and goals for tuberculosis control. 2. Epidemiology of TB and DR-TB: Assess the prevalence of tuberculosis and drug-resistant tuberculosis in the specific setting to determine the testing needs. 3. Diagnostic network structure and capacity: Evaluate the existing diagnostic network structure and capacity to determine the feasibility of implementing and maintaining the selected tests. 4. Facility and infrastructure requirements: Consider the infrastructure and facility requirements for implementing the tests, including laboratory equipment, trained personnel, and quality assurance measures. 5. Implementation considerations: Take into account the practical aspects of implementing the tests, such as cost-effectiveness, scalability, and sustainability. 6. Testing site demand: Determine the specific testing needs at the selected molecular rapid diagnostic test sites, including whether the test is needed for initial detection of TB alone or for detection of resistance to specific TB medicines [23].

These factors should be assessed in a stepwise process to identify the most suitable molecular rapid diagnostic tests for tuberculosis in a specific setting.

Centralized molecular assays refer to high-throughput diagnostic tests that are performed in centralized laboratories rather than at the point-of-care. These assays use molecular techniques to detect the presence of *Mycobacterium tuberculosis* (the bacterium that causes tuberculosis) and to identify resistance to rifampicin and isoniazid, two important drugs used in tuberculosis treatment.

The functioning of centralized molecular assays involves several steps. First, a respiratory specimen, such as sputum, is collected from the patient suspected of having tuberculosis. The specimen is then processed in the laboratory to extract the genetic material (DNA) of the bacteria. This DNA is then amplified using a technique called polymerase chain reaction (PCR), which makes multiple copies of specific regions of the DNA. Next, the amplified DNA is analyzed using specific probes or primers that target regions of the *M. tuberculosis* genome associated with tuberculosis and drug resistance. These probes or primers can detect the presence of *M. tuberculosis* and identify specific genetic mutations that confer resistance to rifampicin and isoniazid.

The results of the assay are typically reported as positive or negative for tuberculosis, as well as indicating the presence or absence of drug resistance mutations. These results can help guide appropriate treatment decisions for patients with tuberculosis as they are rapidly available.

Overall, centralized molecular assays offer a high-throughput and accurate method for diagnosing tuberculosis and detecting drug resistance. They are performed in centralized laboratories, which may have the advantage of processing a large number of samples efficiently. However, it is important to ensure that there are reliable systems in place to transport specimens and deliver test results to patients in a timely manner for these assays to have a significant impact on patient care.

In the past, World Health Organization (WHO) had recommended the use of nucleic acid amplification tests (NAATs) for the detection of tuberculosis (TB) and drug resistance. In 2020 WHO advocated for universal testing for both rifampicin and isoniazid resistance before initiating TB treatment and recommended four additional moderately complex tests [22,23].

In the systematic review and meta-analysis, the BD Max MDR-TB assay showed a sensitivity of 93% (95% CI 89.0-96.0) and a specificity of 97% (95% CI 96.0-98.0) on raw sputum specimens. For decontaminated sputum specimens, the sensitivity was 91% (95% CI 87.0-94.0) and specificity was 95% (95% CI 93.0-97.0). In comparison to the Xpert MTB/RIF assay, the BD Max MDR-TB assay had similar sensitivities of 91% and 90% and specificities of 96% and 98%, respectively [24].

Attribute	Abbott	BD Max	Bruker Hains	Roche
detect	a. MTBC b. INH+RIF	MTB+INH+RIF	a. MTBC b. INH+RIF	a. MTBC b. INH+RIF
Target/s	IS 6110, pab geneRRDR of rpoB + INH promotor + kat G	IS 6110, IS 1081, both multi-copy+ devR + RRDR of rpoB + INH promo-tor+ katG 315	IS 6110 RRDR of rpoB +inh A+ kat G	16SrRNA+ esx RRDR of rpoB+INH promotor+katG
LOD	17 cfu/ml 60 cfu/ml for DST	0.5 cfu/ml 6 cfu/ml for DST	15 cfu/ml 20 cfu/ml for DST	7.6 cfu/ml 8.8 cfu/ml for DST
Sample prepa-ration	Automated, 4.5 hrs	Manual, 1.5 min/ sample	automated	Automated
PCR	Automated RealTime PCR	Automated multiplex PCR	Automated RealTime PCR	Automated RealTime PCR
IQC	+ & - controls	In every cartridge	+ & - controls	+ & - controls
Tests perrun	96	24	96	96, 384

Figure 1

The sensitivity and specificity of the new methods for detecting isoniazid (INH) and rifampicin (RIF) susceptibility varied across the different assays. Here are the sensitivity and specificity ranges for each assay:

Abbott RealTime RIF/INH assay:- Sensitivity for RIF resistance: 94% (95% CI 89.0-99.0)- Specificity for RIF resistance: 100% (95% CI 99.0-100) - Sensitivity for INH resistance: 89% (95% CI 86.0-92.0) - Specificity for INH resistance: 99% (95% CI 98.0-100).

FluoroType MTBDR assay:- Sensitivity for RIF resistance: Range: 97%-99%- Specificity for RIF resistance: Range: 100% (95% CI 85.0-100)- Sensitivity for INH resistance: Range: 70%-92% - Specificity for INH resistance: Range: 100% (95% CI 84.0-100).

BD Max MDR-TB assay:- Sensitivity for RIF resistance: 90% (95% CI 55.0-100)- Specificity for RIF resistance: 95% (95% CI 91.0-97.0)- Sensitivity for INH resistance: 82% (95% CI 63.0-92.0) - Specificity for INH resistance: 100% (95% CI 98.0-100).

A Multicentric study found that the BD MAX system had high sensitivity and specificity for detecting TB and drug resistance, making it a potentially valuable tool for rapid identification of TB globally. BD MAX system was evaluated for its ability to detect mutations associated with resistance to rifampin (RIF) and isoniazid (INH), which are two common drugs used to treat tuberculosis. The study specifically looked at mutations in the rpoB and katG genes, as well as the inhA promoter region, which are known to be associated with multidrug-resistant TB [25].

The BD MAX system is an automated, qualitative in vitro diagnostic test that is used for the detection of Mycobacterium tuberculosis complex (MTBC) and mutations associated with resistance to rifampin (RIF) and isoniazid (INH) in patients suspected of having tuberculosis (TB). It offers several advantages in TB diagnostics: 1. Rapid and accurate detection: The BD MAX system provides a quick turnaround time, with results available in less than 4 hours. It has a high sensitivity and specificity for the detection of both MTBC and drug resistance mutations, making it a valuable tool for the rapid identification of TB cases. 2. Comprehensive coverage: The BD MAX system can detect mutations in the rpoB and katG genes, as well as the inhA promoter region associated with multidrug-resistant TB (MDR-TB). This comprehensive coverage allows for the identification of a wide range of drug resistance patterns, enabling individualized therapy for patients. 3. Automation and integration: The BD MAX system is fully automated and integrated, reducing the need for manual handling and minimizing the risk of human error. It requires a stable source of electricity and laboratory technician training, making it suitable for use in central laboratories where large numbers of specimens are tested. 4. Suitable for high-TB-burden settings: The BD MAX system has been evaluated in low- and middle-income settings with a high burden of TB. Its performance has been found to be comparable to other molecular diagnostic tests, such as Xpert MTB/RIF [25,26].

Recently it has been reported from Botswana that Gene Xpert failed to detect a variant with Rifampicin resistance. Use of Next Generation Sequencing (NGS) found the isolate was a pre XDR with resistance to INH, Ethambotol, pyrazinamide, moxifloxacin and levofloxacin besides having rifampicin resistance due to rpoB 1491 F mutation [27].

A clinical trial has been initiated to address the question, Is INH essential in the treatment of TB? The key findings of the study suggest that isoniazid may not be essential during the first 14 days of tuberculosis therapy. The study found that omitting isoniazid from standard multidrug therapy for the first 14 days did not have a deleterious effect on lowering sputum bacterial burden. The results were consistent whether evaluated by colony forming unit (CFU) counts or liquid culture time to positivity (TTP). This is surprising because isoniazid is known to have potent killing activity against rapidly dividing bacteria during the initial days of therapy. However, the study did not evaluate long-term clinical outcomes, so further research is needed to determine the overall effectiveness of isoniazid in tuberculosis treatment [28].

In view of the fact that majority of isolates found in India are still DS TB (78%) and would lead to high rule rate (85%), rapid simultaneous detection of MTBC along with INH and Rif susceptibility followed by initiation and completion of standard treatment would contribute towards rapid cure of the patient as well elimination of tuberculosis by decreasing spread of infection. Similarly identification of INH or Rif monoresistance or MDR TB should lead to suitable modification thus limiting their spread as has been the experience in France [29].

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